OVERALL GROWTH OF TOMATO (*Lycopersicon esculentum* L. cv. Glacier) INOCULATED WITH SPECIES OF *GLOMUS* AND *TRICHODERMA* GROWING UNDER GREENHOUSE CONDITIONS

By

Brian J. Adkins

Horticulture and Crop Science Department

California Polytechnic State University

San Luis Obispo

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ABSTRACT

The overall plant growth of tomato (*Lycopersicon esculentum* L. cv. Glacier) cultivated in greenhouse conditions along with inoculation treatments of beneficial fungi species were observed. This study included 2 brand name products containing species of fungi which include: 1)*Botanicare Guardian TR* containing *Trichoderma harzianum* 10,000,000 CFU/g, *T. hamatum* 10,000,000 CFU/g*, T. koningii* 10,000,000 CFU/g; and 2)*Botanicare Guardian MY* containing *Glomus intraradices* 57 propagules/g; *G. fasiculatum* 57 propagules/g; *G. etunicatum* 57 propagules/g; *G. clarum* 57 propagules/g. Treatments include: Treatments: 1) 2g *Botanicare Guardian TR*; 2) 2g *Botanicare Guardian MY*; 3) 1g *Botanicare Guardian TR* + 1g *Botanicare Guardian MY;* and 4) Control. The overall height increase was 55.04%, 49.36%, 44.42% for treatments 3, 2, 1 respectively. The overall Fresh Weight increase was 50.47%, 40.95%, 33.55% for treatments 3, 2, 1 respectively. The overall dry weight increase was 62.67%, 53.23%, 42.60% for treatments 3, 2, 1 respectively. These findings are consistent with other studies displaying a synergistic effect between various species of fungi. Compatible combinations of various species of AM and *Trichoderma,* which result in cropping systems that fully utilize AM and *Trichoderma* symbiosis, are the most efficient, sustainable, and environmentally sound large scale methods for food production. Further study of synergistic affects of AM and *Trichoderma* on plant growth, and should be conducted to ensure fungal species are compatible with each other and are compatible for a given a given crop.

TABLE OF CONTENTS

Section Page

I. Introduction……………………………………………………………………………………………………….1

II. Literature Review………………………………………………………………………………………………3

Abiotic Disorders……………………………………………………………………………………………3

Environmental Concerns…………………………………………………………………………………..4

Arbuscular Mycorrhizal (AM) Fungi…………………………………………………………………….5

Phosphorous Uptake by AM Fungi……………………………………………………………………..5

Nitrogen Uptake by AM Fung……………………………………………………………………………7

Calcium Uptake by AM Fungi……………………………………………………………………………7

Water Uptake by AM Fungi………………………………………………………………………………8

Trichoderma and Nutrient/Water Uptake……………………………………………………………9

III. Materials and Methods……………………………………………………………………………………11

IV. Results and Discussion……………………………………………………………………………………13

Treatment vs. Height……………………………………………………………………………………13

Treatment vs. Fresh Weight……………………………………………………………………………14

Treatment vs. Dry Weight………………………………………………………………………………14

Conclusion……………………………………………………………………………………………………16

V. References…………………………………………………………………………………………………….18

INTRODUCTION

In the life of tomato plant, there are many challenges within the environment to overcome; these include infectionous diseases from bacteria, fungus, and viruses, or other physical damage from insects that continuously feed on the foliage. Another major problem for tomato plants is the availability of essential nutrients necessary for proper plant growth and development of reproductive organs such as the flowers and fruit. These deficiencies can not only be caused by the lack of nutrients in the media, but also if the nutrients needed for plant growth exceeds the roots ability to uptake nutrients that are available when rapid growth is occurring. Tomato plants can also be subject to water stress that can cause reduced plant vigor.

Normally, when plants are showing signs of stress from nutrient deficiencies, we add more fertilizers and chemicals are added to try to correct the problem. But what if something passive not active can prevent, not correct, this problem of deficiencies and reduce the overall use fertilizers and chemicals used to grow tomatoes? If plants are water stressed, then logically, more water needs to be supplied, but what is the optimal rate for plant growth as water is becoming more scarce and expensive, and is there a way to be more efficient using this water?

It has been known for a long time that beneficial microorganisms within the soil form symbiotic relationships with the roots of plants, which in turn, enhance the uptake of essential nutrients by plants. This enhancement allows the nutrients already in the soil to become more available for plant use, reducing the need to add extra fertilizers or chemicals, and also assist in preventing, not correcting, deficiency problems. These microorganisms also have the ability to uptake water, as they act as extensions of the roots.

Currently, there are a few companies that manufacture beneficial microorganisms, so any homeowner or commercial grower has easy access to these products. The main species of beneficial microorganisms produced are species of *Trichoderma* and Mycorrhizal fungi, and both can be used in soil or soilless media. This is important, as the tomato industry not only grows in the field, but also in greenhouses and using soilless media such as Rockwool. Taken as a whole, the benefits of improved nutrient uptake can be observed by comparing overall height, fresh weight and dry weight of plants of various treatments. The purpose of this experiment is to evaluate the overall growth of tomato (*Lycopersicon esculentum*) plants inoculated with various species of *Trichoderma* and Mycorrhizal fungi.

LITERATURE REVIEW

A large number of factors, both environmental and cultural practices, affect the ability of tomato (*Lycopersicon esculentum* L. cv. Glacier) to uptake nutrients effectively and efficiently. Nutrient deficiencies are the most common in very acid or very alkaline soils due to the immobilization of nutrients at the lower and higher soil pHs. Some soils are naturally low in specific nutrients due to their composition, and excessive, or unbalanced, use of fertilizer may also cause some nutrients to be less available (Gabor, 1997). Low or excessive moisture content may also affect nutrient availability, as this water is the carrying medium for minerals in the plant from the root hairs to every plant part. Water is the also the medium in which all chemical reactions in the plant occur, and is a reagent in many of the most important reactions such as photosynthesis and respiration (Hartmann et al., 1988).

Abiotic Disorders

As (Gabor, 1997) describes, all nutrient deficiencies lead to some sort of abiotic disorder, ex: stunting of growth from lack of Nitrogen (N), Phosphorous (P) deficiency turning leaves purple with eventual senescence of those leaves and necrosis of foliage from the insufficiency of Calcium (Ca). On tomato, Ca deficiency can also lead to the disorder Blossom-End-Rot, but water imbalances can provide stresses that evoke this destruction of fruit, which means excessive soil salinity and root damage can be factors leading to a water disproportions. Blossom-End-Rot symptoms begin as light tan lesions turning to a dark brown sunken area at the blossom end of the fruit accompanied by a dark rot (Gabor, 1997). Although P may be present in the media, it may be immobile or unavailable for plant uptake. P is a structural of nucleic acids to build DNA and RNA, and is also the energy bearing molecule ATP, is a component in some enzymes catalyzing metabolic reactions (Hartmann et al., 1988), and is in the initial reactions of photosynthesis. If P becomes limited, root growth is suppressed with few fibrous roots, so plant growth is limited, resulting in susceptibility to damage by root rot fungi. Overall, P deficiency symptoms begin with short, thin shoots, leaves are small, and defoliation follows starting with the lower leaves. It is commonly known that N is the element most likely to be deficient. Nitrogen may be lost from the soil to the atmosphere by the process denitrification that converts nitrate to gaseous compounds of nitrogen (Ludwick, 1998). Nitrogen, in the nitrate form, is also lost due to its high mobility, which can lead to easily being leached. Since N is part of all amino acids that make up all of the proteins and protoplasts, is required in enzymes, and the chlorophyll molecule (Hartmann et al., 1988), N is essential to the growth of plants. Chlorophyll synthesis cannot occur without N, so plants that are deficient become chlorotic beginning with the old foliage and growth is reduced resulting in stunting and thin, weak shoots.

Environmental Concerns

Besides abiotic symptoms, tomatoes are also susceptible to many fungal diseases which cause infection through the roots. These fungi include species of *Pythium, Phytophthora, Rhizoctonia,* and *Fusarium* just to name a few. Whether trying to control nutrient deficiencies or trying to control pathogenic diseases, by breaking the disease cycle (Agrios, 2005), applying chemical fertilizers or fungicides can lead to destruction to our environment. These hazardous chemicals have the ability to leach into the groundwater, eventually ending up in water supply, which causes harm to humans consuming that water (Ludwick, 1998).

Alternate solutions using beneficial microorganisms are now being studied to control nutrient deficiencies, while at the same time control fungal diseases. These studies have shown that species of Arbuscular Mycorrhizal (AM) and *Trichoderma* fungi have the ability to increases plant growth, increase yield, decrease water stress, decrease salt stress, and reduce infections from fungal diseases (Hamel et al., 2007).

Arbuscular Mycorrhizal (AM) Fungi

AM fungi have coevolved with plants and soil for over 400 million years to become part of the root system of a very large number of terrestrial plant species (Taylor et al., 1995). The extra radical mycelium of AM fungi is one of the key features of AM symbiosis (Agrios, 2005). It is largely responsible for the uptake function of mycorrhizae and for translocation of nutrients and water from the soil to the plant (Hamel et al., 2007).

Phosphorous (P) Uptake by AM Fungi

As a result of strong P fixation power of soils, the size of the available P pool is not sufficient to ensure maximum crop yields, as only 15% or less of P fertilizers are used by crops during the year of application, while the remaining P is absorbed, fixed, or retrograded (Hamel et al., 2007). Plant species fulfill their P requirements using different strategies: rapid root growth, root elongation, root hair proliferation, and modifications of rhizosphere conditions (Smith, 2001). In addition, AM roots have external mycelia that can be considered an extension of the roots. As described by (Smith, 2003) in the direct pathway, high-affinity plant P transporters located in the epidermis and root hairs are involved in uptake of orthophosphate from the soil solution directly into plant cells. If the rate of uptake exceeds the rate of diffusion of P in the soil solution, the concentration of P is reduced leading to 1- to 2-mm zones of depletion close to the root surfaces, which limit the rate of uptake. The mycorrhizal pathway involves uptake of P from the soil solution by AM fungal transporters located in external hyphae. P is then translocated rapidly over considerable distances (1-15 cm) and is delivered to fungus-plant interfaces in the root cortex. Plant P transporters located at these interfaces absorb P into root cortical cells. The efficiency of external hyphae at acquiring P from soil solution is related to their small diameter (<10μm) compared to that of roots and root hairs, which reduces the distance of P ion diffusion and the formation of P depletion zones (Hamel et al., 2007). The alleviation of P depletion zones allows for continuous uptake during the growing period. The thin hyphae also proliferate extensively and penetrate smaller pores in the soil than roots do, resulting in a larger root system. A study conducted by (Cavagnaro, 2006) showed that AM tomato shoots had 75% increase in P uptake and the fruit had 41% increase in P uptake, while other reports attribute 70-80% of plant P to AM fungal uptake and translocation (Smith and Read, 1997).

Nitrogen (N) Uptake by AM Fungi

Some studies have shown that AM mycelia can absorb and translocate large amounts of N to the host plant (George, 1995). Nitrogen uptake by AM is more complex than P uptake as both plant roots and AM hyphae can take up N easily, plant available N exist in more than one form, and N influences the development of both AM mycelia and roots. Both forms, ammonium (NH_4^+) and nitrate (NO_3^-) can be used by plants, but the NH_4^+ binds onto soil particles and is therefore less readily available than NO_3^-. It has been shown that AM fungi can contribute significantly to plant N nutrition in soil where NO_3^- dominates by increasing plant NO_3^- uptake, the percentage of plant N derived from fertilizer, and plant use efficiency (Azcón, 2001). AM fungi have been found to metabolize NH_4^+ as well, through glutamine synthetase activity (Smith et al., 1985). AM fungi have improved plant uptake of N from complex organic materials, and the decomposition of organic residues can be enhanced by the presence of AM fungi (Hodge, 2001). This may indicate that, although plant roots may not always need AM fungi to take up glycine, they may sometimes benefit from the enhancing effect of AM fungi on organic residue decomposition. The capture of N from organic sources has sometimes been related to root length, but it has also been related to extraradical hyphae length when root growth has been contained in divided growth containers (Hodge, 2001).

Calcium (Ca) Uptake by AM Fungi

Results have shown that AM hyphae can absorb and transport Ca, and in general, when the availability of Ca is low, AM fungi enhance plant uptake of Ca (Hamel et al., 2007).It is generally accepted that Ca uptake is a passive process associated with the uptake of water as Ca moves into the root down an electrochemical potential gradients and is confined primarily to the apoplastic pathway (Orloff, 1984). Consequently, under low soil moisture conditions relative cations such as K and Na concentrations in the soil solution increase, enhancing their uptake while lowering the electrostatic attraction required for the uptake of Ca in not-AM plants.

Water Uptake by AM Fungi

Several mechanisms are involved in the reduction of water stress in AM plants and are related to the extraradical AM mycelia (Hamel et al., 2007). AM hyphae can absorb water as demonstrated by the difference in transpiration in plants with and without extraradical hyphae (Hardie, 1985). Some studies concluded that the enhanced drought tolerance provided by AM fungi is probably due to drought avoidance rather than to a change in the ability of leaves to withstand dehydration (Davies et al., 2002). The enhanced ability of AM plants to absorb water is related to the length of their extraradical mycelia, and (Azcón, 2003) found that different AM fugal species depleted soil water to different degrees, as soil water uptake was related to the abundance of soil hyphae. Extraradical AM hyphae may also improve the capacity of a root system to extract soil water by giving it access to micropore water, because of their small diameter; hyphae can enter pores that are too small for root hairs to access. Furthermore, AM hyphae proliferate well beyond the limit of root hairs, giving plants access to more water-filled pores (Hamel et al., 2007). The ability of AM plants to extract more soil water may lead to better nutrition of N and Ca during times of water stress. (Subramanian and Charest, 1998) found higher activities of nitrate reductase, glutamine synthetase, and glutamate synthase in AM plants after a period of water stress. They also found total amino acids, soluble proteins, and total N content were higher in AM plants, suggesting better N nutrition for AM plants under water stress. (Subramanian et al., 2006) found AM plants had significantly higher uptake of N and P in both roots and shoots regardless of intensities of drought stress, and AM inoculation also significantly increased shoot dry matter and the number of flowers and fruits. Furthermore, AM plants produced tomato fruits that contain significantly higher quantities of ascorbic acid and total soluble solids (TSS) and AM effects increased with increasing intensity of drought.

Trichoderma and Nutrient/Water Uptake

It has been documented that several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants and those various mechanisms include antibiosis, parasitism, inducing host-plant resistance, and competition. But what is not well known is the ability of *Trichoderma* to uptake nutrients and water to enhance overall plant growth in the same approach as the plants inoculated with AM. In most instances, increased plant growth and yields were attributed to the reduction in plant disease the biological control agents, but *Trichoderma harzianum* has been reported to increase plant growth independent of any plant disease (Baker et al., 1984). (Rudresh, 2005) found the increased growth, P uptake, and yield parameters in *Trichoderma*-inoculated treatments compared with fertilized control and rock phosphate control treatments suggest that *Trichoderma* spp. solubilize insoluble rock phosphate and supply P in a soluble form to plants in addition to acting as a biological control agent and possibly producing growth-promoting substances. Among the *Trichoderma* isolates, *T. harzianum* was found to be the best isolate whose performance was just behind the standard phosphate-solubilizing bacterium, and (Kleifeld, 1992) reported similar observations of an increase in growth and yield parameters by inoculation with *Trichoderma* species. Some studies have also shown that *Trichoderma* spp. can stimulate the growth of a number of vegetable and bedding plant crops (Baker, 1988) from their results, and those of (Ousley et al., 1994); they concluded that specific *Trichoderma* strains have the potential to consistently increase plant growth. (Naseby, 2000) has found that inoculation with *Trichoderma* strains significantly increased the wet shoot weight by 15%, significantly increased the root weights by 22%, and significantly greater wet root weights up to 62%.

MATERIALS AND METHODS

The plant species tested is Tomato (*Lycopersicon esculentum* L. cv. Glacier) inoculated with the 2 brand name products: containing *Trichoderma harzianum* 10,000,000 CFU/g, *T. hamatum* 10,000,000 CFU/g*, T. koningii* 10,000,000 CFU/g and *Botanicare Guardian MY* containing *Glomus intraradices* 57 propagules/g; *G. fasiculatum* 57 propagules/g; *G. etunicatum* 57 propagules/g; *G. clarum* 57 propagules/g. The tomatoes plants were started by sowing 120 seeds in a transplant media containing 2 parts fir bark, 1 part peat moss, 1 part #2 perlite. The seeds were placed in a propagation greenhouse containing glass glazing with 85°F day and 75°F night temperatures. The mist runs for 30 seconds every 10 minutes from 9am-5pm. Transplant 60 seedlings that are 3 in. in height 14 days after sowing seeds by first filling 60 #1 nursery containers with a 1-1-1 media containing 1 part fir bark, 1 part peat moss, 1 part #2 perlite and 30 oz. dolomite/cu. Yd. for pH adjustment. Second, 15 #1 nursery containers were dibbled and Treatment 1 (T) was applied= 2g *Botanicare Guardian TR*/container by sprinkling inside dibbled hole, then 15 #1 nursery containers were dibbled and Treatment 2 (M) was applied= 2g *Botanicare Guardian MY*/container by sprinkling inside dibbled hole, then 15 #1 nursery containers were dibbled and Treatment 3 (T/M) was applied= 1g *Botanicare Guardian TR* + 1g *Botanicare Guardian MY*/container by sprinkling inside dibbled hole, and the Treatment 4 (C) was the control for the remaining 15 #1 nursery containers which were only dibbled, for a total of 4 treatments and 15 replicates of each treatment. Then a 3in. seedling was placed in each #1 nursery container. Following transplanting and inoculation (application of treatments 1, 2, and 3), the plants were placed in an acrylic glazed greenhouse with 75°F day and 65°F night temperatures and spaced according to the random experimental design, Fig.1.

R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
T	T/M	C	M	T	T/M	C	M	T	T/M	C	M
M	T	T/M	C	M	T	T/M	C	M	T	T/M	C
C	M	T	T/M	C	M	T	T/M	C	M	T	T/M
T/M	C	M	T	T/M	C	M	T	T/M	C	M	T
T	T/M	C	M	T	T/M	C	M	T	T/M	C	M

Fig. 1. This is the random experimental design using 1 sq. ft. spacing. T= Treatment 1, M= Treatment 2, T/M= Treatment 3, and C= Control.

The plants were watered using regular water; everyday for the first week, then everyday following. 28 days after transplanting seedlings, the height in cm was measured from the soil line. Each plant was also harvested at the soil line and weighed in grams (fresh weight) using a digital scale and placed into individual paper bags to dry. After drying for 36 hours inside the paper bags, each plant was reweighed (dry weight).

Statistical Analyses of Data:

Minitab 15, one-way analysis of variance (ANOVA) was used to analyze all data. Critical differences at the 5% level of significance were tested using Fisher's least significant difference test.

RESULTS AND DISCUSSION

Treatment vs. Height

Overall growth of plants can be measured to some degree by the total height, as the theory of a larger plant will have larger yields than smaller plants of the same species. In this study, Treatment 2 had a significantly larger height than Treatment 1, which is to be expected, as AM is well documented to increase plant size (Hamel et al., 2007). What is surprising is the fact that Treatment 3 (combination of AM and *Trichoderma*) was significantly taller (p-value<0.001) than all other treatments, suggesting a synergistic effect of the fungi (Table 1). The difference in height could be contributed to an increase in uptake of water with the fungi mycelia acting as an extension of the roots. The overall height increase was 55.04%, 49.36%, 44.42% for treatments 3, 2, 1 respectively.

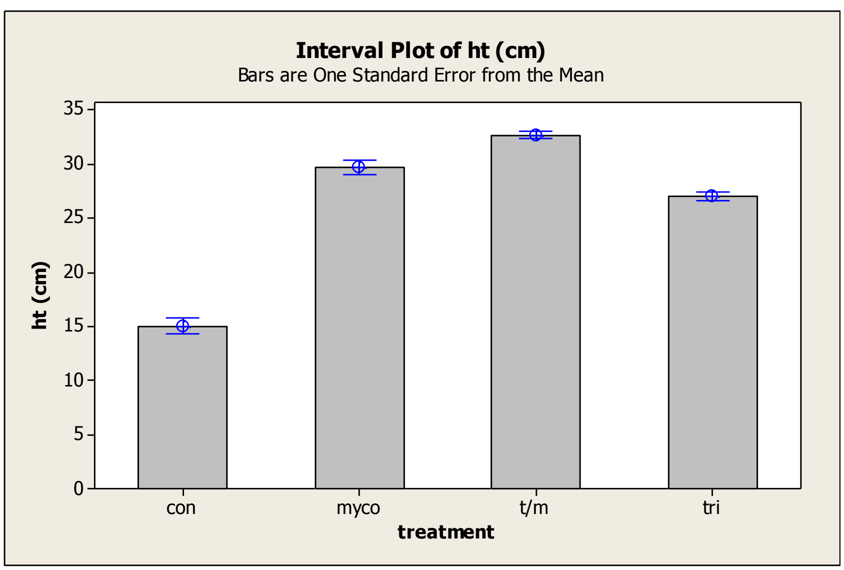

Table 1. Using Minitab 15, one-way analysis of variance (ANOVA) was done on all of the Height vs. Treatment data measured in this experiment. Critical differences at the 5% level of significance were tested using Fisher's least significant difference test.

Treatment vs. Fresh Weight

By looking at fresh weight measurements, a significant difference (p-value<0.001) in fresh weight between all treatments was observed, as once again Treatment 3 had the largest overall mean, while having 2-fold the fresh weight of the control (Table 2). The overall Fresh Weight increase was 50.47%, 40.95%, 33.55% for treatments 3, 2, 1 respectively. Once again this could be contributed to the fungi mycelia acting as an extension of the roots enabling the plant to uptake more water.

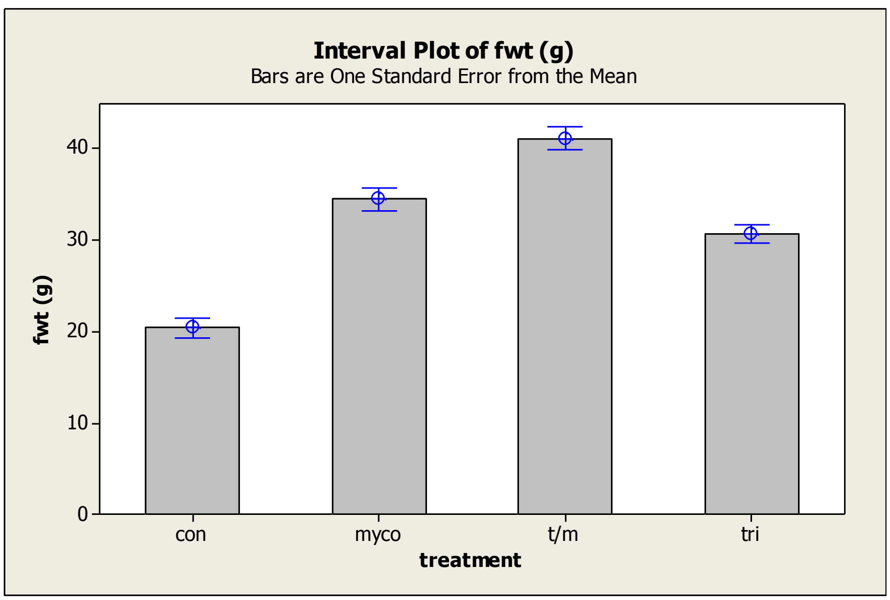

Table 2. Using Minitab 15, one-way analysis of variance (ANOVA) was done on all of the Fresh Weight vs. Treatment data measured in this experiment. Critical differences at the 5% level of significance were tested using Fisher's least significant difference test.

Treatment vs. Dry Weight

The overall dry weight, again, is an example of the possible synergistic effects between species of *Glomus* and *Trichoderma*, as Treatment 3 was significantly different (p-value<0.001) from all other treatments. The dry weight was measured 36 hours following fresh weight measurements (Table 3). The overall dry weight increase was 62.67%, 53.23%, 42.60% for treatments 3, 2, 1 respectively.

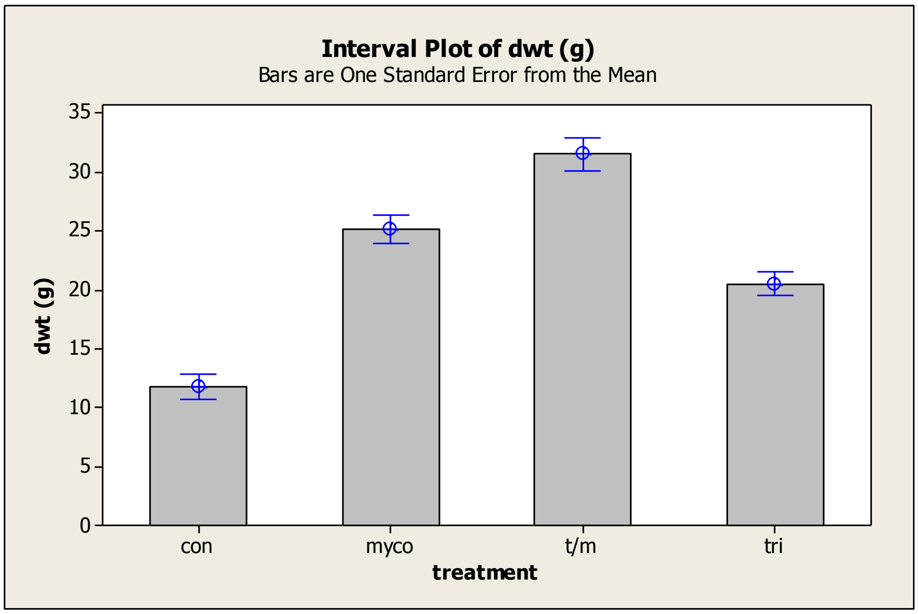

Table 3. Using Minitab 15, one-way analysis of variance (ANOVA) was done on all of the Dry Weight vs. Treatment data measured in this experiment. Critical differences at the 5% level of significance were tested using Fisher's least significant difference test.

In all treatments, the control was significantly lower when comparing height, fresh weight, and dry weight, suggesting that the inoculation of AM and *Trichoderma*, whether alone or together, had major influences on tomato plant development. This could be contributed to an increase of water uptake by the plant, as no extra fertilizers were applied. There were no significant data differences between: height vs. fresh weight; height vs. dry weight; or fresh weight vs. dry height.

These findings are consistent with other studies displaying a synergistic effect between various species of fungi as (Siddiqui, 2008) showed that *T. harzianum* increased tomato plant growth by 18%, while *T. harzianum* along with G. intraradices increased plant growth by 37%. (Srinath, 2003) studied the influence of *G. mosseae* inoculated with *T. harzianum*, and plant showed maximum plant height, biomass, P content, mycorrhizal root colonization, AM fungal spore numbers, and increased populations of *T. harzianum* in the root zone when the organisms were inoculated together. While (Gamalero, 2004) found the highest values of root architecture parameters were recorded in plants inoculated with the three microorganisms: total root length, total root surface area and number of tips were synergistically increased, while an additive effect was observed on total root volume and degree of root branching. Longer and more-branched root systems could be considered more efficient both in soil exploration and in water uptake and transport, favoring the successful establishment of many plant species. Another study (Gaur, 2003) showed the co- inoculation of *G. Mosseae, G. constrictum,* and *G. fasciculatum* produced the best growth stimulation in greenhouse grown tomatoes.

Conclusion

AM fungi have the ability to naturally increase water uptake, which in turn can lead to increased plant growth, yield, and still have moderate biocontrol affects on pathogens. While *Trichoderma* fungi greatly reduces infections from pathogens, but only has moderate increase in plant water uptake, growth, and yield, for the best results, a compatible combination of various species of AM and *Trichoderma* fungi should be the considered. AM and *Trichoderma* fungi are an essential component of natural soil-plant. While it is possible to design systems devoid of beneficial organisms, as in greenhouse production, it is clear that cropping systems that fully utilize AM and *Trichoderma* symbiosis are the most efficient, sustainable, and environmentally sound large scale methods for food production. AM and *Trichoderma* fungi can extract soil water efficiently, allowing good crop yields to be produced from soils or media with limited fertility, thus are essential for cropping systems, such as organic farming, that have a low impact on the environment. Advances in molecular biology techniques have made it possible to develop diagnostic tools to estimate potential contribution of microorganisms in a soil to a given crop. This routine testing of microorganisms' potential will lead to more accurate fertilizer recommendations and the safe reduction of fertilizer inputs, which will translate into reduced chemical seepage into the environment. Further study of synergistic affects of AM and *Trichoderma* on plant growth, and should be conducted to ensure fungal species are compatible with each other and are compatible for a given a given crop.

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